## REMARKS

Claims 1 to 34 are pending of which claims 33 and 34 are new. Support for these new claims can be found in the paragraph bridging pages 19 and 20 and in the first paragraph of page 24. Any amendments to the remainder of the claims set forth herein were not intended and do not limit the scope of the present invention.

In paragraph 1, the Office vacated the previous Office Action in view of a non considered preliminary amendment mailed shortly prior to that Office Action but not received by the Examiner in time. Applicants would like to thank the Examiner for reconsidering this application in view of the preliminary amendment.

In paragraph 2, the Office noted that the inventors' declaration is defective as it identifies the priority claim to provisional application 60/462,289 as being made under 35 USC §119(a)-(d) and 365(b).

In response applicants submit a new inventors' declaration in which priority to this provisional application is appropriately claimed under 35 USC §119(e).

Also in paragraph 2, the Office objected to the specification as missing a heading for the "Brief Description of the Drawings."

In response, the appropriate amendment has been made.

In paragraph 3, the Office rejected claims 1 to 27 under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

In particular, the Office rejected selected claims for having insufficient antecedent basis for the terms (a) "said nucleic acid molecule" (claims 1 to 27), (b) "said amplified nucleic acid" (claims 1, 9, 12 and 24) and "said label" (claims 7 and 22).

Applicants submit with regard to (a) that in the preliminary amendment submitted on February 21, 2006 in claim 1, line three "acid" was inserted between "nucleic" and "molecule." Applicants respectfully submit that this amendment addressed the antecedent basis issue raised by the Office. Similarly, applicants submit that the amendment to claims 7 and 22 in the same preliminary amendment addressed the issue raised under (c). With respect to (b), applicants have further amended claim 1,

subparagraph (b) and claim 12, subparagraph (b) to insert a specific reference to "amplified nucleic acid" to provide further antecedent basis.

In paragraph 4, the Office objected to claims 1 to 14 and 19 to 32 under 35 USC §103(a) as being obvious over U.S. Patent 6,251,594 to Gonzalgo et al. (hereinafter "Gonzalgo") in view of U.S. Patent 6,258,568 to Nyren et al. (hereinafter "Nyren").

The Office expressed the view that Gonzalgo teaches all elements of claim 1, but for a real time sequencing step to determine the methylation status at a predetermined nucleotide position in the sample. However, the Office reasoned that Nyren discloses such a real-time sequencing step.

As stated in the abstract, Gonzalgo determines the strand-specific methylation status of cytosine residues via a bisulfite treatment of DNA followed by a methylation-sensitive single nucleotide primer extension (Ms-SnuPe). In this context, applicants would like to direct the Office's attention to applicant's disclosure in the paragraph bridging pages 16 and 17 and the examples of the specification where the method of the present invention is compared with SnaPshotTM, the non-radioactive version of Gonzalgo's Ms-SnuPe.

Nyren describes a method for pyrosequencing.

To establish a prima facie case of obviousness, there must be (1) some suggestion or motivation, either in Gonzalgo and/or Nyren or in the knowledge generally available to one of ordinary skill in the art, to combine the reference teachings. (2) There must also be a reasonable expectation of success. Finally, (3) the references, when combined, must teach or suggest all the claim limitations (MPEP §2142).

Initially applicants note that the Office states in the paragraph bridging pages 4 and 5 that Nyren teaches "a method wherein real-time sequencing is performed to determine the methylation status at a predetermined nucleotide position." Applicants submit that Nyren does not mention DNA methylation anywhere in his disclosure. In fact, Nyren goes into quite some detail of what biological phenomena can be detected using his sequencing method (column 13, line 59 and following), but does not mention DNA methylation. This is of particular interest since, at the time Nyren filed his application, DNA methylation was a widely discussed phenomenon.

Applicants further note that independent claim 1 requires:

"(c) real-time sequencing said amplified nucleic acid treated with said agent" (emphasis added)

Independent claims 12 and 32 contain similar language.

In the paragraph bridging pages 7 and 8 of the specification, the term "*real-time sequencing*" is defined as "*sequence analyses which allow specific sequencing, i.e. determination of the sequence of a nucleic acid molecule in real-time* (emphasis added)."

Applicants submit that using Gonzalgo's DNA methylation detection method in combination with Nyren's sequencing method would not result in "real-time sequencing" according to the present invention.

In particular, the use of Gonzalgo's method even with the benefit of the disclosure of Nyren would require an extension of the 3' end of a primer with only a single, directly labeled, ddNTP and the evaluation of the methylation status of only this single base. Thus, while it could be argued (though applicants do not support or concede the veracity of such an hypothetical argument), that the methylation status of Gonzalgo's single base could be assessed "in real time", the "determination of the sequence" of the nucleic acid is not in real time as required by the presently claimed invention.

Accordingly, a combination of Gonzalgo and Nyren does not teach or suggest all elements of the invention as presently claimed and as required for a *prima facie* case of obviousness (MPEP §2142).

Applicants' further note that Gonzalgo's stated quest is to provide a method for determining DNA methylation using familiar procedures suitable for widespread use (column 3, lines 57 to 62). He approaches this quest by providing a method in which a DNA strand is only extended by one nucleotide and methylation is only detected at the respective base that is added. Gonzalgo method includes separation of radiolabelled product, for example, by electrophoresis on polyacrylamide gels (column bridging cols. 4 and 5). Nyren describes an improved rapid sequencing method that, among others, eliminates the need for an electrophoresis step.

As outlined above, applicants have established that not every element of the independent claims is disclosed by a combination of Gonzalgo and Nyren and that thus no *prima facie* case of obviousness has been established. However, applicants submit

that even if Gonzalgo and Nyren would disclose every element of the claimed invention, which applicants deny, it is well established that the mere identification in the prior art of each element is insufficient to defeat the patentability of the combined subject matter as a whole. Rather, to establish a *prima facie* case of obviousness based on a combination of elements disclosed in the prior art, the Office must articulate the basis on which it concludes that it would have been obvious to make the claimed invention. *In re Kahn,* Fed. Cir. 2006, 04–1616. The Office's basis is stated in the paragraph bridging pages 5 and 6 of Office Action. It appears that the Office argues that the person skilled in the art would be motivated to combine Gonzalgo and Nyren because the combination promises to be faster and more cost efficient.

Applicants note that this type of motivational analysis would render a number of methods attractive for combination with Gonzalgo. However, Gonzalgo specifically dismisses a number of those methods (background section) and advances his method in view of its reliability.

Thus, Gonzalgo provided little motivation to combine his method with a high throughput method such as the one of Nyren. Nyren, on the other hand, discloses that his method can be used for the detection of single base changes, DNA polymorphisms and rare point mutations. He does not mention that his method is useful for characterizing epigenetic phenomena such as DNA methylation. In addition, Nyren discloses as substrate for his sequencing method only native DNA and cDNA. He does not suggest using chemically modified DNA. This is despite the fact that the importance of DNA methylation had been discussed fairly intensively in the literature at the time Nyren filed his application.

Applicants further note that the reaction mixture used in Nyren is considerably more complex than that used in Gonzalgo's identification method, which uses next to the DNA, essentially just a polymerase, a specific ddNTP and a primer. Nyren uses all nucleotides for primer extension, employs a nucleotide-degrading enzyme, a $PP_i$ detection enzymes (e.g., ATP sulfyrase and luciferase) and a nucleiosid-5'-phosphosulfate (e.g., APS) for the formation of sulfyrase substrate and luciferin as substrate for the luciferase. Thus, there a three further enzymes including their substrates in the Nyren's reaction mixture. Applicants submit that the increase of complexity in the reaction mixture and in particular the effect of the three additional enzymes on the remainders of the substances introduced by the chemical modification

and vice versa would further discourage a person skilled in the art to combine the references.

Thus, applicants submit that even if the combination of Gonzalgo and Nyren would disclose all elements of the presently claimed invention, which applicants submit it does not, there is no explicit or implicit motivation to combine the references.

Claim 10 requires:

"further comprising quantifying the methylated nucleotides."

New claim 34, which depends from claim 10 requires:

"wherein an allele frequency of 5% can be detected."

This ability to detect an allele frequency of 5% reflects the high sensitivity of the method of the present invention. Nowhere in Gonzalgo or Nyren is such a high degree of sensitivity suggested or disclosed.

In fact Nyren notes at a number of places that his method is prone to substantial background noises (column 7, lines 48 to 55: since nucleotide dATP is a substrate for apyrase; column 10, lines 5 to 6: in view of the digestion of 3' ends by proof-reading-polymerases; column 10, lines 16 to 19: accumulation of final products during sequencing cycles; column 12, lines 38 to 41: due to the contamination of, for example, dNTP-charged with $PP_i$, see also Figs. 3 and 4). Applicants submit that such a propensity to background noise renders it unlikely that with Nyren's method the sensitivities of the invention expressed by claim 34 could be accomplished. Background is particularly disturbing if the base distribution of the sequence subject to sequencing does not correspond to a 50/50 distribution, which is common in DNA methylation. In this context, applicants note that while Nyren mentions quantitative detection of signals (column 10, lines 49 to 62), he refers here only to a discrimination at an allele distribution of 50% (column 10, lines 49 to 53).

Applicants have shown above, in addition to not disclosing all elements of the claimed invention, that there is also not the required motivation to combine Gonzalgo and Nyren for a *prima facie* case of obviousness.

In addition, to name only a few of numerous praises that the present invention has obtained, applicants notes that the analysis of DNA methylation using real-time

sequencing has been named the "gold standard for quantitative DNA-methylation analysis" (see attached Biotage write up "Pyro-software"). The same write up also cites Dr. Triantafillos Liloglou from the University of Liverpool Cancer Research Centre as saying that "Quantitative DNA methylation analysis by Pyrosequencing enabled us to distinguish tumor-specific methylation from "noise." Also, Dupont et al. noted that "among the numerous technologies for methylation analysis, pyrosequencing represents a breakthrough" Analytical Biochemistry, 2004, 333, p.119-127 (attached "Dupontetal"). Further laudatory statement about the method do exist and the undersigned will be happy to provide them to the Office upon request.

The Biotage website also provides evidence for the invention's apparent commercial success: Over this website CpG-Methylation laboratory services are offered (attached "CpGMeth-lab-service"); as well as different PyroMark® RUO tests (attached "PyromarkRUOtest") and Pyro Q-CpG software (attached "Pyro-software").

Thus, even if a *prima facie* case of obviousness would have been established, what applicants deny, this evidence of "secondary" consideration provides compelling evidence that the invention is non-obvious (MPEP §2141).

In paragraph 5, starting on page 12 of the Office Action, the Examiner rejects claims 15, 16, and 18 as obvious over Gonzalgo in view of Nyren and further in view of U.S. Patent 5,786,146 to Herman.

Some of the deficiencies of Gonzalgo and Nyren have been outlined above. Applicants respectfully submit that Herman does not cure these deficiencies.

In addition, applicants note that neither Gonzalgo nor Nyren teach the diagnosis of neurodegenerative diseases or neurological disorders (compare discussion of claim 3 on page 6 of the Office Action). Hermann cures neither this deficiency nor the other deficiencies of Gonzalgo and Nyren discussed above.

In paragraph 6, starting on page 13 of the Office Action, the Office rejected claim 17 under 35 USC §103(a) as being unpatentable over Gonzalgo in view of Nyren and further in view of U.S. Patent Publication 2003/0232351 to Feinberg.

As noted in the context of the previous rejection, neither Gonzalgo nor Nyren teach the diagnosis of neurodegenerative diseases or neurological disorders (compare discussion of claim 3 on page 6 of the Office Action). Feinberg cures neither this deficiency nor the other deficiencies of Gonzalgo and Nyren discussed above.

Applicants have shown above that independent claims 1, 12 and 32 are non-obvious in view of Gonzalgo when combined with Nyren. These claims should therefore

be in condition for allowance. Claims 2 to 11, 13 to 31 and 33 to 34 that are dependent therefrom should also be in condition for allowance.

The undersigned sincerely urges the Office to call her at the number provided below to discuss any issues that might arise in the further prosecution of this case.

The fee for a one-month extension of time as well as for two additional claims over 20 is submitted herewith. No further fees are believed to be due. However, the Commissioner is authorized to charge or credit deposit account no. 50-3135 as required.

Respectfully submitted,

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